Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1365–1370

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Methionyl Adenylate Analogues as Inhibitors of Methionyl-tRNA Synthetase

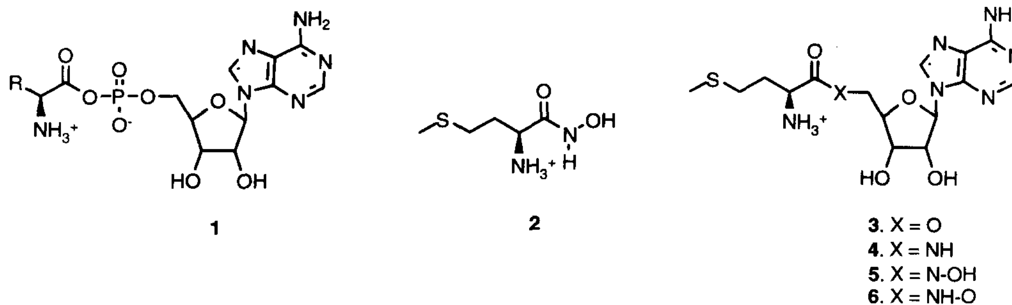
Jeewoo Lee,[1]* Sang Uk Kang,[1] Mee Kyoung Kang,[1] Moon Woo Chun,[1] Yeong Joon Jo,[2] Jin Hwan Kwak,[3] Sunghoon Kim[4]

[1]Laboratory of Medicinal Chemistry, College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, Korea [2]ImaGene Co. 608-2, Jaeil-Rhi, Yangji-Myun, Yongin, Kyunggi-Do 449-820, Korea [3]School of Bioscience and Food Technology, Handong Univerisity, Namsong-Ri 3, Buk-Ku, Pohang 791-949, Korea [4]Korea National Creative Research Initiatives Center for ARS Network, Sung Kyun Kwan University, 300 Chunchun-Dong, Jangan-Ku, Suwon, Kyunggi-Do 440-746, Korea

Received 8 February 1999; accepted 1 April 1999

Abstract: Four stable analogues of methionyl adenylate (**3-6**) were designed as inhibitors of methionyl-tRNA synthetase and synthesized from 2',3'-isopropylideneadenosine. They strongly inhibited aminoacylation activity of methionyl-tRNA synthetases isolated from *Escherichi coli, Mycobacterium tuberculosis, Saccharomyces cerevisiae* and human. Among the microorganisms tested, however, these chemicals showed the growth inhibition effect only on *E.coli.* © 1999 Elsevier Science Ltd. All rights reserved.

Aminoacyl-tRNA synthetases (aaRSs) are universal and essential for all living organisms. The enzymes catalyze the transfer of a particular amino acid to its corresponding specific tRNA to form each aminoacyl-tRNAs, which are used for protein synthesis by the matching the tRNA anticodon with its cognate amino acid, resulting in faithful translation of genetic information.[1] Their use as drug targets can be accomplished by finding selective inhibitors of pathogen synthetases to human cell counterpart. The inhibitors against these enzymes have been attracted much interest as new antimicrobial agents, especially to overcome the development of widespread resistance to mainline antibiotics.[2,3] Most aaRSs catalyze aminoacylation reaction in two steps. In the first step, the amino acid is activated by nucleophilic attack on ATP at the α-phosphate to form an aminoacyl adenylate (**1**, aminoacyl AMP or aaAMP) and pyrophosphate. In the second step, the activated amino acid is transferred to the 3'-terminal ribose of a cognate tRNA that directs its placement within a growing polypeptide chain. Two different mechanisms for the latter step classify aaRSs into two classes in which, for class I enzymes, the aminoacyl group of aaAMP is transferred initially to the 2'-OH of the terminal adenylate in tRNA, then moved to the 3'-OH by a transesterification, while for class II, the aminoacyl group is transferred directly to the 3'-OH.[4,5]



0960-894X/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(99)00206-1

Since the aminoacyl adenylate, a mixed anhydride intermediate generated during the reaction, is bound much more tightly to the enzyme than substrates, amino acid and ATP, generally by two or three orders of magnitude, structural analogues based on the intermediate have the potential to be tight binding inhibitors to the enzymes. Several aminoacyl adenylate analogues have thus been designed and synthesized in the following forms: 1) phosphonate analogues[6] for alanyl-tRNA, lysyl-tRNA and phenylalanyl-tRNA synthetase, 2) ester analogues for glutamyl-tRNA synthetase,[7] 3) sulfamate analogues for alanyl-tRNA,[8] isoleucyl-tRNA,[9] prolyl-tRNA,[10] and seryl-tRNA synthetase[11]. The aim is to obtain tight binding and stable inhibitors by replacing the labile anhydride bond of aminoacyl adenylate with stable non-hydrolyzable bioisosteres. Pseudomonic acid (mupirocin), which is currently the only drug used as an aaRS inhibitor, is also regarded as a type of aminoacyl adenylate analogues because it occupies both of the isoleucine and ATP binding site for the selective inhibition of bacterial isoleucyl-tRNA synthetase.[12]

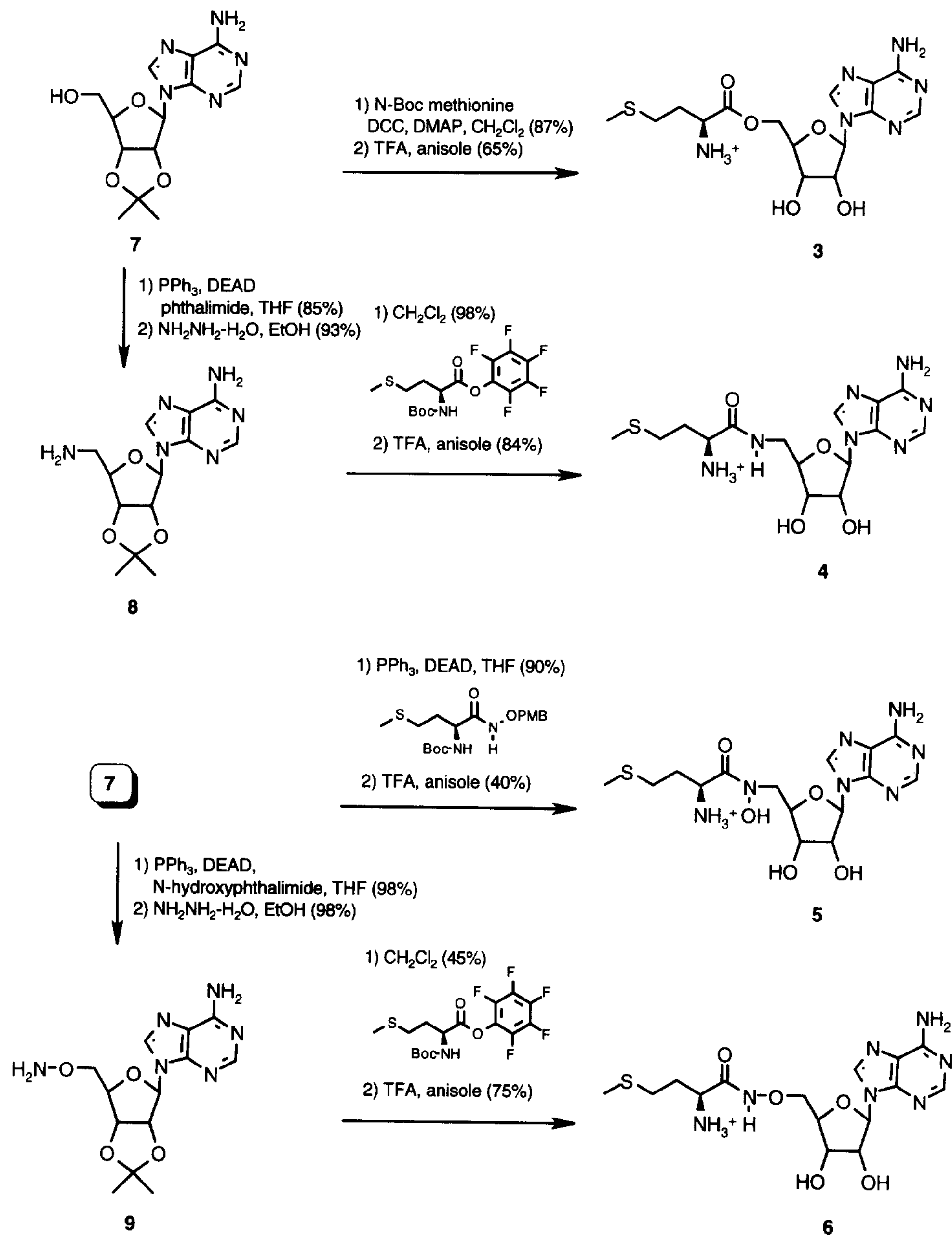
Recently, we reported that methionine analogues were synthesized as inhibitors of *E. coli* methionyl tRNA synthetase (MetRS) and L-methionine hydroxamate (**2**) was found to have the most potent enzyme inhibitory and antibacterial activities.[13] As part of our continuing effort to find potent and pathogen-selective inhibitors of methionyl-tRNA synthetases as novel antibacterial agents, we herein describe the syntheses and biological activities of methionyl adenylate analogues as MetRS inhibitors.

Design and Synthesis of Methionyl Adenylate Analogues

The design of most aminoacyl adenylate analogues reported thus far has been based on replacing the labile acylphosphate linkage of the intermediate with its stable bioisostere such as ester, phosphonate, sulfamate or sulfonamide. In particular, sulfamate group, having similar polar interactions with the enzyme as compared to acylphosphate of aaAMP by X-ray analysis,[11] have been well-studied and have proven to be useful as its surrogate, resulting from good inhibition to the corresponding aaRS.[8-11] As inhibitors of MetRS, we initally synthesized ester (**3**) and amide (**4**) analogues of methionyl adenylate. Since L-methionine hydroxamate (**2**) was found to be a good inhibitor of MetRS as presented in previous report,[13] the attachment of adenylate to it was expected to increase inhibitory activity by additional binding of adenosine to the enzyme. Thus, N-alkyl hydroxamate (**5**) and O-alkyl hydroxamate (**6**) analogues, respectively, were also synthesized.

Ester analogue **3** was prepared from 2',3'-isopropylideneadenosine **7**, which was commercially available, by coupling with N-Boc-methionine following by deprotection under acidic condition in high yield. For the synthesis of amide analogue **4**, the 5'-hydroxyl group of **7** was converted into the corresponding amine **8** by Gabriel synthesis. The amine of **8** was condensed with N-Boc-methionine pentafluorophenol ester to give the adduct cleanly and the protecting groups were removed by acidic protocol. Two hydroxamates, **5** and **6**, were synthesized by similar approaches. The Mitsunobu reaction of **7** with N-Boc-O-(4-methoxybenzyl)methionine hydroxamate, which was prepared by coupling between N-Boc-methionine and O-4-(methoxybenzyl)hydroxylamine hydrochloride, gave the adduct in good yield and was subjected to acidic deprotection to produce N-hydroxamate analogue **5**. For the synthesis of O-hydroxamate analogues **6**, adenosine **7** was converted into O-adenosylhydroxylamine **9** by the Mitsunobu reaction with N-hydroxyphthalimide followed by hydrazine reaction. The amine of **9** was converted to the final product by the same method as that used for the synthesis of **4**. The structures of all target compounds were confirmed by spectral analysis.[14]

Scheme



Biological Results and Discussion

The enzyme inhibitory activity of the synthesized compounds was determined by measuring the decrease of the reaction product, the [^{35}S]methionylated *E. coli* tRNA^{fMet}, in the presence of 270 μM of each chemical, after carrying out aminoacylation reaction by *E. coli* methionyl-tRNA synthetase.[13] Their inhibitory activities to the enzyme have been depicted in percent compared with the control condition in which the enzyme carried out the reaction in the absence of inhibitors as shown in Figure 1. L-methionine hydroxamate (**2**) was used as a standard for comparing relative potency. Four methionyl adenylate analogues showed significant inhibitory activities to the enzyme and their activities were more potent than hydroxamate (**2**). Ester analogue (**3**) was found to be the most potent compound in this series and their relative activities were in the following order: **3** (ester) > **5** (N-hydroxamate) > **4** (amide) > **6** (O-hydroxamate) ≈ **2**. The estimated K_i of **3** and **5** represented 10.9 μM and 13.1 μM, respectively. Similarly, glutamic acid esters were previously reported as surrogates of glutamyl adenylate for inhibition of *E.coli* glutamyl-tRNA synthetase.

The main issue for developing aaRS inhibitors into antimicrobial agents is whether they have sufficient selectivity for the pathogen synthetase and don't interfere with the enzyme in human cells. In order to examine their species-selective inhibition of synthesized compounds, they were thus tested against MetRSs from three other different organisms including *Mycobacterium tuberculosis*, yeast and human cells. As can be seen by the results presented in Figure 2, all compounds inhibit the enzyme in human cells as well as in pathogens to similar extent. Even though O-hydroxamate (**6**) was relatively selective for pathogens, its potency was still moderate. Recently, structure activity relationship studies of isoleucyl adenylate analogues targeting IleRS indicated that structural modification of adenine moiety endowed them with species-selectivity[9], probably because the binding site of adenine ring in synthetases is presumed to be more variable and affordable between species than other sites. Such modification of adenine ring with our potent compounds is under progress and may eventually overcome their selectivity problems.

Antibacterial activities of methionyl adenylate analogues (**3-6**) were examined against eight different bacterial species by the agar dilution method[15]; their minimal inhibitory concentration (MIC) values are represented in Table. They showed a strong growth inhibition to *E.coli* and moderate inhibition to *B. cereus*. However, the extent of their activities was proportionally correlated to the extent of enzyme inhibition. Since excellant *in vivo* antibacterial activity, despite relatively low in vitro efficacy, was found in isoleucyl adenylate analogues[9], further screening *in vivo* will be carried out in these compounds.

In conclusion, we synthesized stable bioisosteres of methionyl adenylate, a tight-binding intermediate of the enzyme reaction, as inhibitors of methionyl-tRNA synthetase. Its ester and N-hydroxamate analogues (**3** and **5**) were found to be potent inhibitors of *E. coli* MetRS and inhibited the growth of *E. coli*. However, they did not discriminate MetRS of different species. Further structure activity relationship studies will be directed toward increasing the species-selectivity of the lead compounds.

Acknowledgement

This study was supported in part by a grant (# HMP-97-D-4-0014) of the '97 Good Health R&D Project, Ministry of Health & Welfare, and by a grant of National Creative Research Initiatives from Ministry of Science and Technology, R.O.K.

Figure 1. Relative inhibitory activities of L-methionine hydroxamate (**2**) and methionyl adenylate analogues (**3-6**) toward *E.coli* methionyl-tRNA synthetase

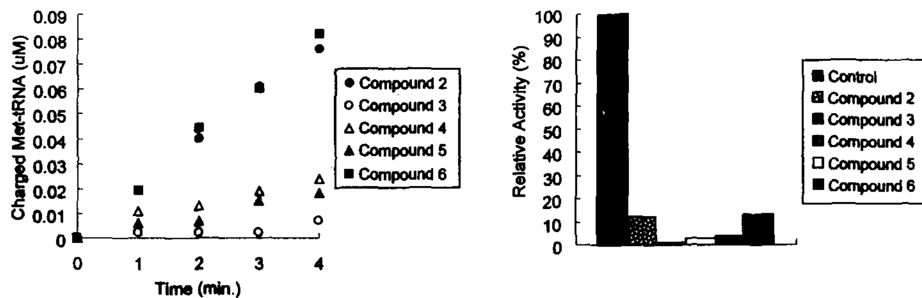


Figure 2. Relative inhibitory activities of methionyl adenylate analogues (**3-6**) toward four different methionyl-tRNA synthetases from *E.coli*, *Mycobacterium tuberculosis*, yeast, and human cells.

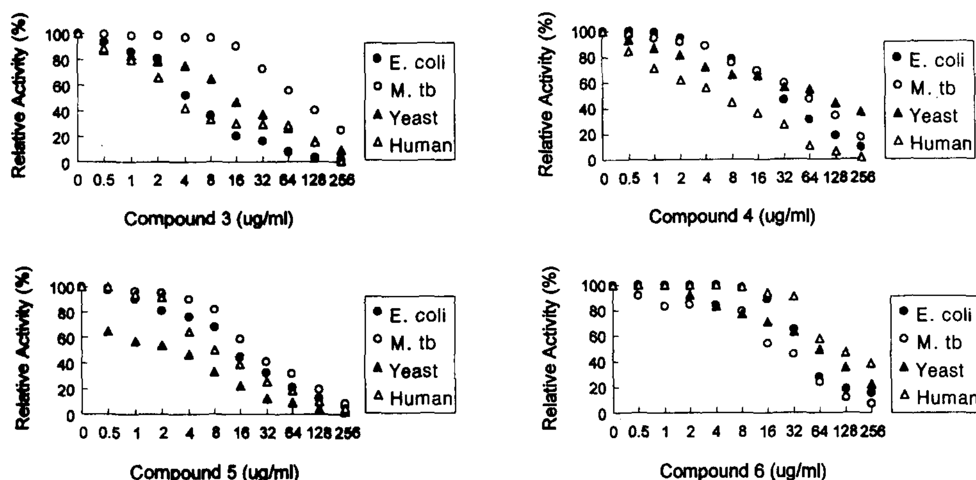


Table . Antibacterial activities of methionyl adenylate analogues (3-6)

	MIC(μg/ml)				
Microorganisms	**2**	**3**	**4**	**5**	**6**
Staphylococcus aureus 1538p	>64	>64	>64	>64	>64
Streptococcus epidermidis 887E	>64	>64	>64	>64	>64
Bacillus cereus ATCC 27348	>64	32	>64	8	>64
Escherichia coli JM109	0.5	0.5	1	0.5	8
Salmonella typhimurium 14028	8	>64	>64	>64	>64
Klebsiella pneumoniae 2011E	>64	>64	>64	>64	>64
Klebsiella aerogenes 1976E	4	>64	>64	>64	>64
Pseudomonas aeruginosa 1912	>64	>64	>64	>64	>64

References and Notes

1. van der Haar, F.; Gabius, H. J.; Cramer, F. *Angew. Chem. Int. Ed. Engl.* **1981**, 20, 217.
2. Schimmel, P.; Tao, J.; Hill, J. *FASEB J.* **1998**, 12, 1599.
3. Renau, T. E.; Hecker, S. J.; Lee, V. J. *Annual Reports in Medicinal Chemistry* **1998**, 33, 121.
4. Arnez, J. G.; Moras, D. *TIBS* **1997**, 22, 211.
5. Cramer, F.; Freist, W. *Angew. Chem. Int. Ed. Engl.* **1993**, 32, 190.
6. Biryukov, A. I.; Zhukov, Y. N.; Lavrik, O. I.; Khomutov, R. M. *FEBS* **1990**, 273, 208.
7. Desjardins, M.; Desgagnés, J.; Lacoste, L.; Yang, F.; Morin, M-P.; Lapointe, J.; Chênevert, R. *Bio. Med. Chem. Lett.* **1997**, 7, 2363.
8. Ueda, H.; Shoku, Y.; Hayashi, N. Mitsunaga, J.; In, Y.; Doi, M.; Inoue, M.; Ishida, T. *Biochim. Biophys. Acta* **1990**, 1080, 126.
9. Hill, J. M.; Yu, G.; Shue, Y-K.; Zydowsky, T. M.; Rebek, J. US Patent 5,726,195.
10. Heacock, D.; Forsyth, C. J.; Kiyotaka, S.; Musier-Forsyth, K. *Bioorg. Chem.* **1996**, 24, 273.
11. Belrhali, H.; Yaremchuk, A.; Tukalo, M.; Larsen, K.; Berthet-Colominas, C.; Leberman, R.; Beijer, B. ; Sproat, B.; Als-Nielsen, J.; Grübel, G.; Legrand, J-F.; Lehmann, M.; Cusack, S. *Science* **1994**, 263, 1432.
12. Yanagisawa, T.; Lee, J. T.; Wu, H. C.; Kawakami, M. *J. Biol. Chem.* **1994**, 269, 24304.
13. Lee, J.; Kang M. K.; Chun, M. W.; Jo, Y. J.; Kwak, J. H.; Kim, S. *Bio. Med. Chem. Lett.* **1998**, 8, 3511.

14. **Compound 3:** $C_{15}H_{22}N_6O_5S$-CF_3COOH

    a stick solid; $[\alpha]^{20}_D$ =-12.6 (c 0.25, MeOH); ^{1}H NMR (D_2O) δ 8.32 (s, 1 H, H-8), 8.24 (s, 1 H, H-2), 6.10 (d, 1 H, *J* = 4.9 Hz, H-1'), 5.01 (t, 1 H, *J* = 4.6 Hz, H-2'), 4.5-4.7 (m, 2 H, H-3' and H-4'), 4.48 (m, 2 H, H-5'), 4.23 (t, 1 H, *J* = 6.6 Hz, C<u>H</u>COO), 2.4-2.6 (m, 2 H, SC<u>H</u>$_2$CH$_2$), 2.08-2.16 (m, 2 H, SCH$_2$C<u>H</u>$_2$), 1.92 (s, 3 H, CH$_3$S); ^{13}C NMR (D_2O) 172.8, 155.7, 151.8, 143.3, 117.3, 91.1, 84.2, 75.8, 75.0, 68.4, 54.5, 31.8, 31.0, 16.6; FAB MS *m/z* 399 (MH$^+$)

    Compound 4: $C_{15}H_{23}N_7O_4S$-CF_3COOH

    a stick solid; $[\alpha]^{20}_D$ =-16.0 (c 0.75, MeOH); ^{1}H NMR (D_2O) δ 8.33 (s, 1 H, H-8), 8.24 (s, 1 H, H-2), 6.06 (d, 1 H, *J* = 6.1 Hz, H-1'), 4.94 (dd, 1 H, *J* = 4.9, 6.1 Hz, H-2'), 4.28-4.35 (m, 2 H, H-3' and H-4'), 4.08 (t, 1 H, *J* = 6.6 Hz, C<u>H</u>CONH), 3.87 (dd, 1 H, *J* = 8.0 and 14.2 Hz, H-5a'), 3.52 (dd, 1 H, *J* = 3.6 and 14.2 Hz, H-5b'), 2.3-2.45 (m, 2 H, SC<u>H</u>$_2$CH$_2$), 1.95-2.1 (m, 2 H, SCH$_2$C<u>H</u>$_2$), 1.89 (s, 3 H, CH$_3$S); ^{13}C NMR (D_2O) δ 172.4, 158.4, 155.7, 151.8, 143.3, 117.8, 90.5, 85.3, 75.8, 74.3, 55.3, 44.3, 32.9, 30.7, 16.7; FAB MS *m/z* 398 (MH$^+$)

    Compound 5: $C_{15}H_{23}N_7O_5S$-CF_3COOH

    a stick solid; $[\alpha]^{20}_D$ =-27.3 (c 0.5, MeOH); ^{1}H NMR (D_2O) δ 8.26 (s, 1 H, H-8), 8.07 (s, 1 H, H-2), 6.01 (d, 1 H, *J* = 5.9 Hz, H-1'), 4.76 (t, 1 H, *J* = 5.6 Hz, H-2'), 4.44 (dd, 1 H, *J* = 3.4 and 5.1 Hz, H-3'), 4.31 (m, 1 H, H-4'), 3.8-4.0 (m, 3 H, H-5' and C<u>H</u>CONOH), 2.67 (t, 2 H, *J* = 7.6 Hz, SC<u>H</u>$_2$CH$_2$), 2.1-2.3 (m, 2 H, SCH$_2$C<u>H</u>$_2$), 2.15 (s, 3 H, CH$_3$S); ^{13}C NMR (D_2O) δ 177.1, 158.3, 155.3, 151.1, 143.4, 121.8, 91.2, 88.6, 76.6, 73.5, 64.4, 56.8, 32.6, 30.7, 16.8; FAB MS *m/z* 414 (MH$^+$)

    Compound 6: $C_{15}H_{23}N_7O_5S$-CF_3COOH

    a stick solid; $[\alpha]^{20}_D$ =-9.7 (c 0.5, MeOH); ^{1}H NMR (D_2O) δ 8.39 (d, 1 H, *J* = 2.6 Hz, H-8), 8.25 (d, 1 H, *J* = 2.6 Hz, H-2), 6.10 (d, 1 H, *J* = 4.1 Hz, H-1'), 4.79 (t, 1 H, *J* = 4.3 Hz, H-2'), 4.49 (t, 1 H, *J* = 5.4 Hz, H-3'), 4.40 (m, 1 H, H-4'), 4.22-4.30 (m, 2 H, H-5'), 3.88 (m, 1 H, CHCON), 2.38-2.45 (m, 2 H, SC<u>H</u>$_2$CH$_2$), 1.93-1.99 (m, 5 H, SCH$_2$C<u>H</u>$_2$ and CH$_3$S); ^{13}C NMR (D_2O) δ 169.0, 154.8, 151.4, 150.4, 144.9, 117.4, 91.6, 85.4, 79.0, 76.7, 73.1, 53.2, 32.6, 31.1, 16.9; FAB MS *m/z* 414 (MH$^+$)

15. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 3rd Edition; Approved Standard M7-A3. NCCLS, Villanova, PA. **1993**.